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The Basic Components of the Cuckoopint

(Arum maculatum L.)

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A steam volatile basic fraction was obtained from fresh leaves of Arum maculatum L. In addition to nicotine (0.7 mg/kg of the natural drug) it contained traces of another alkaloid and larger amounts of ethyl-,isobutyl- and isoamylamine. Non-volatile alkaloids could not be detected in the drug, but there were traces of non-volatile primary amines.

The statement is repeatedly made in the literature that in the spotted cuckoopint in addition to the "acid tasting component" a "basic principle" is present which is partly called an alkaloid. In connection with the problem whether this uncertainty is due to the presence of "Chemical Species", it seemed necessary, first of all, to examine the basic fraction more closely.

Bird[1] was the first one to report a volatile base in Arum maculatum in 1885. During the same year , Spica and Biscaro[2] made the same finding for Arum italicum, which could not be confirmed by other writers. Thereupon Chauliguet and coworkers[3] concerned themselves in detail with the isolation of the volatile base from A. maculatum. Using a well known process, they were able to obtain 4-5 grams of a dark base from 100 kilograms of fresh herb. Judging from the odor reminding them of mouse urine and the volatility, they assumed that a conine or similar alkaloid was involved and that this was identical with the "acid principle". Subsequently, Steiner and Stein von Kamienski[4] reported only recently on the detection of ethyl- and isobutylamine in the spadix of A. italicum. A short time ago Simon[5] found isobutylamine in the same organ of A. maculatum.

1-Taken from source cited in footnote 3
2- G. Spica and G. Biscaro, Gazz chim. ital. 15 , 238 (1885).
3- J. Chauliaguet, A. Herbert and F. Heim, Compt. rend. 124, 1368 (1897).
4-M. Steiner and E. Stein von Kamienski, Naturwissenschaften 40, 483 (195
5-E.W. Simon, J. exp. Bot. 13, 1 (1962).

Isolation and characterization of the bases

The isolation process worked out by Cromwell for the chromatographic microdetermination of conium alkaloids seemed to be indicated in this case also. First of all, it was tested with hemlock, and in some details the process was carried out with greater care. Accordingly, 401.2 mg brown crystals with an unpleasant conine-like odor could be obtained from 10 kg of fresh leaves of A. maculatum (habitat: Kaiserslautern). Thus we were apparently dealing with product identical with, or at least similar to that of Chauliaquet and coworkers. Then a part thereof was tested paper chromatically for conium alkaloids under the Cromwell conditions. thereupon and after using different separating layers and solvent systems, we arrived at the definite result that no conine or gamma coniceine was present in our basic fraction. To be sure, the chromatograms showed two Dragendorff and iodoplatinate zones, the main zone of which corresponded with nicotine in each case. As for the secondary alkaloid present in a considerably smaller amount, it was also certain that one of the hemlock alkaloids was not involved, judging from its position and the color reagents. It is still worth mentioning that our basic fraction did not reveal the typical needle prick reaction of the natural drug on the tongue; thus, an "acid tasting reaction" is out of the question.

a) Qualitative and quantitative nicotine detection

It had to be assumed already after comparing the size of the spots chromatographed together with known amounts of nicotine that the basic fraction was composed of nicotine and the secondary alkaloid only to the smallest degree. Consequently, additional chromatographic and physical identification procedures were taken into consideration. The acetate buffered paper and solvent agent suggested by Walke and Leiserson[7] for the paper chromatography of tobacco alkaloids proved to be particularly favorable. With this solvent, nicotine acid (hRf 15), N-Methylmyosmine (31), Hydroxynicotine (36), Normicotine (41),

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6-B.T. Cromwell, Biochem. J. 64, 259 (1956).

7-T.B. Walker and L. Leiserson, Analyt. Chem, 27, 1129 (1955).

Anabasin (55), Metanicotine (62), Nikotinamide (65), Nicotine (76) and Nikotyrin (94) can be easily separated. Moreover, under these conditions the primary alkaloid showed the same behavoir as nicotine. Now the bromcyan reaction specific for pyridine alkaloids according to Koenig[8] was also carried out on the chromatograms. The primary alkaloid resembled nicotine in color and fluorescence. The secondary alkaloid did not react, and accordingly does not seem to belong in this category. The UV spectra were photographed and evaluated for the further confirmation and microquantitative nicotine determination according to the procedure of Willits and co-workers.[9] Prior to that it had been verified that the amines contained in the basic fraction and the ammonium chloride do not interfere. Since there was no nornicotine according to the chromatograms, the values obtained can be considered realistic. The steam distillate from 20 kg. of fresh leaves had to be used for the quantitative spectrophotometric determination, and resulted in a nicotine content of 14.0 mg, i.e., the freah leaves contain 0.7 mg/kg nicotine.

b) Identification of the volatile amines

The bulk of the basic fraction obtained probably consists of volatile amines. On the paper chromatography set up according to Steiner and Stein von Kamienski[4], the ninhydrinreaction resulted in four easily distinguished colored spots. In order to determine next to which categories the compounds are to be attributed, the reactions mentioned in the experimental part were carried out. According to that, primary alphatic amines are involved. According to the amines chromatographed at the same time for the sake of comparison it could be assumed that ethyl-isobutyl- and isoamylamine were present and the main big spot in the vicinity of the starting point consisted of ammonium salt. Separations on acetate buffered silica Gel G layers with Partridge-mixture gave the same indication.

A part of the basic fraction was reacted with 3,5 Dinitrobenzoylchloride. The 3,5 DNB amides thus obtained again resulted in 3 spots on silica Gel G. with chloroform as solvent, which [spots] lay at the same height as the authentic amine derivatives. Now the basic fraction was separated on paper micro-preparatory. The amines thus obtained individually could then be reacted according to Dihlmann[10] 2.4 Dinitro α naphthol and sublimed accordingly. In a pure state and mixed with the authentic derivatives, the melting points yielded the final identity. The fresh leaves of the cuckoopint contain the isobutyl- and amylamine in approximately the same amounts, while the ethyl amine is present in a somewhat lesser amount.

8- W. Konig, J. pract. Chem. 69, 123 (1904) and 70, 19 (1904).
9- C.O. Willits, M.L.Swain, J.A. Connelly and B.A. Brice Analyt. Chem. 22, 430 (1950).
10-W. Dihlmann, Naturwissenschaften 40, 342 (1953).

c) Non volatile bases (orientating examination)

After nicotine had been detected with certainty in the cuckoopint, the question arose whether cadaverine and putrescine were also present as "biogenic amines" (cf. Hasse).[11,12] Furthermore a presence of Histamine, β -phenylethylamine, choline and acetyl-choline were relevant in relation to the "acid tasting reaction." To begin with , the volatile bases were removed by means of steam distillation for the purpose of isolation. The distillation residue was diluted with phosphor tungstic acid, and the precipitation hereby obtained was worked up and again decomposed. Thereupon the non volatile basic fraction thus obtained could be tested paper chromatographically and thin layer chromatographically. In combination with the color reagents and by means of the reference materials, the result was that this fraction contained 4 non-volatile, primary amines. However, no one was identical to cadaverine or the amines mentioned at the beginning. The small amount of material did not permit any further processing.

Discussion of the Results

The previously existing uncertainty in regard to the presence of bases in the cuckoopint could be cleared up by careful preparation of larger amounts of fresh leaves and the application of micromethods. In connection with the unpleasantly smelling fraction of the volatile bases, we are dealing with a mixture of little nicotine and 3 primary amines. Hemlock alkaloids are not contained with certainty in the drug examined by us. Nicotine as such was already found in a number of plants of the most varying genera. (Blaim)[13]. Even though the amount of nicotine found is very small, the result seems interesting to us nevertheless since this is the first instance in which nicotine was detected in a plant of the genus of the monocotyledons. It is entirely within the realm of possibility that thr tropical araceae contain such alkaloids in larger amounts.

Non volatile alkaloids are, however, not contained in the herb of the cuckoopint. Since none of the basic fractions showed an "acid tasting reaction," we concerned ourselves with this problem also. It appears that a very complex mechanism is present here, and salivary ferments are also involved. It is certain, according to our experiments not cited here, that the araceae acid substance is not identical with the ranunculi acid substances and that the sharp tasting intermediate stages are exceedingly unstable.

Description of the Experiment

Isolation of the unrefined bases

10 kg of fresh leaves (moisture content 92.3%) were ground up coarsely and pulverized with portions of acidified methanol pro analysis (acid concentration 0.1 n HCl) in the Star blender for 5 minutes each. After strong pressing out, the drug residue was again macerated for a few days with acid methanol. The combined

and filtered methanol extracts were reduced in volume to a dry state in a vacuum at room temperature. The dried extract was dissolved 5 times with 100 ml hot water respectively, and the aqueous extract was subjected to a steam distillation after alkalising with sodium carbonate and adding common salt. The distillate caught in n HCl was carefully reduced in volume to approximately 20 ml, and then dried in vacuo in an exsiccator filled with KOH. Yield: 401.2 mg brown crystals.

Thin layer chromatography and paper chromatography

a) For the chromatography a part of the crystals were dissolved in water, alkalized, saturated with common salt, and the free bases shaken out with ether and chloroform. After careful reduction of volume and dissolving in chloroform, this basic fraction could be used directly for chromatography.

b) For the paper chromatography, the paper Schleicher and Schull No. 2043 b in stri-s of 12 X 45 cm was used, and partly subjected to the following preliminary treatment:

α) Cromwell process[6]: Paper laid 15 min. in n HCl, and thereupon the acid washed out quantitatively with water.

Solvents for the paper chromatography: tert. Pentanol + tert. Butanol + n HCl (9+3+2).

β) Impregnation according to Walker and Leiserson[7]: With a mixture of 9.5 ml 0.2 n acetic acid + 90.5 ml 0.2 m sodium acetate solution (pH 5.5).

Solvent: 1- Butanol saturated with acetate buffer pH 5.5.

γ) Impregnation according to Steiner et al: With 0.15 m sodium acetate solution (5 min.).

Solvents: 1-Butanol + glacial acetic acid + water (40 + 10 + 10) and (50+ 1 + 49). For the paper chromatography of the non volatile bases, 2, 4, 6 - Collidin + water (50+50) according to 4, was used as the solvent.

11- K. Hasse and M. Maisack, Naturwissenschaften 42, 627 (1955).
12- K. Hasse and P. Berg, Naturwissenschaften, 44, 584 (1957); cf. K. Mothes, Pharmazie 14, 121 (1959).
13- K. Blaim, Flora 153, 171 (1962).

For the ascending paper chromatography the DESAGA developer camera No. 310 was used, in which the solvent was filled 24 hours before. Before immersing the papers, an equilibrating procudure for one hour took place (temperature approx. 20 degrees). The duration of the paper chromatography was max. 18 hours.

c) For thin layer chromatography work was done according to the Stahl standard method and with column chromatography. In making the layer silica gel G was used preferably with the Merck thin layer chromatograph. The separation of the 3.5 Dinitrobenzamide was best achieved with chloroform + 1 % Ethanol (1) (h Rf values of the DNB of Isoamyl-41 and isobutyl-36 and ethylamine 23).

d) For the visualization of the alkaloids the Dragendorff reagent as modified by Munier and Machboeuf [15] was used. 0.1 g nicotine could still be recognized on silica gel G layers. Iodoplatinate reagent[14] and bromocyanogen[8] was also used. The dried thin layer chromatograms were brought into a vessel with bromcyan vapors for 60 min. for the detection of nicotine, and then sprayed with a 2% 4 Aminobenzoacid ethyl ester solution. Nicotine became yellow in color on silica gel G layers, and orange on aluminum oxide G layers.

e) For the first differentiation of the various amine types, different reagents were used and evaluated as follows: positive reaction (violet red) with ninhydrin reagent; negative with 4 dimethylamino-benzadehyd- diazoniumsalt- folinic- sodium nitroprusside acetaldehyde with isatin reagent.

Quantitative recognition of nicotine

The base hydrochlorides were isolated out of 20 kg fresh leaves in the manner described at the beginning, and shaken out after alkalisation with chloroform. The volume reduced base mixture was then taken up in 50.0 ml 0.005 n HCl, and corresponding dilutions made with 0.05 n n HCl which lay in the optimal area. The spectral photometer PMQ II of the Carl Zeiss Co. was used to photograph the UV spectrum. This photometer has a slit width of 0.02 mm, and the quartz vessels have a layer thickness of 1.001 cm. The spectrum is identical with the nicotine content was calculated according to the log values obtained, and amounted to 14 mg. for the initial drug amount.

3.5 Dinitrobenzamide for Chromatography

250 mg of the base hydrochloride mixture were dissolved in 10 ml water, and coated with a layer of 30 ml ether in a separatory funnel to which was added 0.5 ml pyridin and 0.5 g 3.5 dinitro-benzoylchloride, dissolved in 2 ml Benzol. After adding 11 g sodium carbonate, the aqueous phase was separated after 20 min. and the ethereous layer shaken out with 1% sulfuric acid and water. The ether residue was recrystallized from 50 % ethanol and used in a 5% methanol solution.

2.4 Dinitro naphtol derivatives and microsublimation

50 mg of the base hydrochloride fraction were dissolved in 0.5 ml methanol and applied tape-like on paper (Schleicher and Schull, SaS 2043 b) impregnated with a 0.15m sodium acetate solution for the chromatography. After development (18 hours) a peripheral strip approx. 1 cm wide was cut off from both longitudinal sides of the paper chromatography, and the position of the amines determined by spraying on of ninhydrin reagent. After the concentration the eluates of the corresponding zones of the unsprayed paper chromato-grams were brought each time into a small glass which was surface ground at the top (height 35 mm. aperture diameter 10 mm) and diluted with two drops of 5 n Na 0 11. Every tube was covered for 24 hours with a coverglass which carried on the bottom side a drop of water with a few 2.4 Dinitro α naphthol crystalls suspended therein.

The sublimation of the derivatives formed and the subsequent determination of their melting points took place with the aid of a heating microscope according to Kofler. Authentic ethyl-amine, isobutylamine and isoamylamine were converted in the same way into derivatives.

2.4 DNN derivatives of ethylamine melting point 153-154 degrees, of isobutylamine 148-149 degrees and of isoamylamine 154 degrees.

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